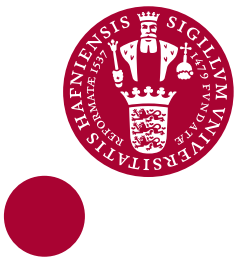

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Pharmacodynamic Modelling of Placebo and Buprenorphine Effects on Event-Related Potentials in Experimental Pain

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Abstract: The purpose of the study was to investigate placebo and buprenorphine effects on event-related potentials (ERPs) in experimental pain and the potential benefit of population pharmacodynamic modelling in data analysis. Nineteen healthy volunteers received transdermal placebo and buprenorphine in a cross-over study. Drug plasma concentrations and ERPs after electrical stimulation at the median nerve with intensity adjusted to pain detection threshold were recorded until 144 hrs after administration. Placebo and concentration-effect models were fitted to data using non-linear mixed-effects modelling implemented in NONMEM (V7.2.0.). Pharmacodynamic models were developed to adequately describe both placebo and buprenorphine ERP data. Models predicted significant placebo effects, but did not predict significant effects related to buprenorphine concentration. Models revealed that ERPs varied both between subjects and between study occasions. ERPs were found to be reproducible within subjects and occasions as population variance was found to be eight times higher than the unexplained variances. Between-subject variance accounted for more than 75% of the population variance. In conclusion, pharmacodynamic modelling was successfully implemented to allow for placebo and variability correction in ERP of experimental pain. Improved outcome of ERP studies can be expected if variation between subjects and study occasions can be identified and described.

Opioids, such as buprenorphine, are widely used in clinical pain management [1], but their usability is often limited by intolerable side effects. Objective tools to improve evaluation of both analgesia and side effects of analgesics are increasingly desirable for clinical pain management and drug development [1]. Several potential tools to evaluate effects of analgesics have been described using experimental human pain models, one of these being electroencephalography [2].

Electroencephalography reflects the electrical activity in the brain with high temporal resolution. The activity may be recorded as event-related potentials (ERPs) on the scalp during repeated stimuli. The shape and timing of ERPs are dependent on the stimulation site and type. Painful electrical stimulation of the extremities in human beings gives rise to a characteristic negative-positive potential in the ERP that can be measured from the vertex position at the scalp [3]. The magnitude of this potential, typically termed the vertex potential has most often been quantified by the amplitude between the visually identified peaks N2 and P2. Previous studies have shown that this vertex potential correlates with the applied stimulus intensity and has been suggested to correlate with pain intensity [3,4]. These and other observations have frequently led to the hypothesis that the vertex potential is produced by signals mediated by the ascending nociception-specific Aδ and/or C-fibres [3]. ERPs may prove valuable in the assessment of effects and side effects of analgesics. Electrically induced vertex ERPs are relatively simple to perform, and they have often been proposed to serve a role as a clinical tool to investigate effects of analgesics [5]. Studies of opioids on vertex ERPs in experimental pain have shown dose and concentration-dependent reductions in ERP magnitude when fixed stimulus intensity has been applied [4,6,7]. However, studies comparing opioids and sedatives suggest that at least part of the opioid-dependent reductions in ERPs are caused by sedation [8,9]. Furthermore, opioid-dependent latency shifts of N2 and P2 have been observed in one study [10], but this has not been reproduced, and the significance of this is unclear.

A major hindrance in the study of ERPs in experimental pain has been very large placebo effects and individual variations in response. Pharmacodynamic non-linear mixed-effects modelling is a technique that allows for mathematical descriptions of placebo effects and drug effects related to plasma concentration and is applicable to studies of pain and analgesics, both across the population and in the individual subjects [11,12]. This allows for the description and explanation of the variability, such as between-subject variabilities (BSV) and between-occasion variabilities (BOV) that can be essential to the evaluation of effects [13]. Despite the promising utility of the pharmacodynamic modelling in the interpretation of analgesic effects on ERPs, only one such study has been found in the literature [14]. However, this was done using a naïve pooled data approach that did not take into account BSV, BOV or placebo effects.

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The hypothesis of this study was that population pharmacodynamic modelling could be used as a tool to improve data analysis of ERP data from experimental pain studies, by accounting for several of the main limitations of traditional ERP analysis. The primary aim was to describe a method to develop pharmacodynamic models to investigate the nature of placebo effects, the distributions of baseline and variations in ERPs for an experimental study in which the applied stimulus intensity was adjusted to achieve the same pain (detection threshold). A secondary aim was to describe how the developed model could be used for placebo-corrected and distribution-normalized analysis of buprenorphine effects on ERP.

Material and Methods

Study design. ERPs were recorded in a randomized, placebo-controlled, cross-over study on 22 healthy male volunteers receiving buprenorphine transdermally, in a dose used for clinical analgesia of 20 μg/hr for 144 hr, as previously described in detail [15]. A third cross-over fentanyl treatment from the same study [15] was not included as it was considered out of scope of the study to develop two models of opioid effects on ERP. The study followed the tenets of the Declaration of Helsinki promulgated in 1964 and was approved by the local Ethical Committee (N-20070061) and the Danish Medicines Agency (EduraCT number: 2007-004524-21), and informed consent was obtained. Pharmacokinetic and pharmacodynamic modelling of psychophysical assessments and different experimental pain models for the study have been published in a complimentary paper [15], and an advanced quantification method of ERP has been published on data [16].

The study included 22 subjects, but two of the subjects dropped out before second arm in the cross-over study. Both received buprenorphine in the first arm. Subjects participated in 7 ERP sessions at pre-treatment and at 4, 24, 28, 48, 72 and 144 hr after the start of treatment. In each session, ERPs were recorded in two replicate runs with approximately 10-min. intervals. Blood samples were collected at pre-treatment, 6, 9, 12, 24, 36, 48, 60, 72, 78, 84, 96, 120, 144, 168, 192 and 216 hr and analysed for plasma concentrations of buprenorphine and norbuprenorphine using ultra-pressure liquid chromatography followed by mass spectrometry detection as previously reported [15].

ERPs were recorded after electrical stimulation of the median nerve. Stimulation was applied at a constant level of pain (pain detection threshold), as opposed to a fixed stimulus intensity as previously reported by several studies [6–10]. This was done to ensure that the cortical processes observed by ERP were actually related to pain. Variation between ERP sessions and treatment with strong analgesic buprenorphine could otherwise lead to stimulation below pain level, which could activate cortical processes very different from those that were aimed to be studied.

Stimuli were applied by an electrical stimulator (Isolator Stimulator Noxi IES 230, JNI Biomedical, Klarup, Denmark) through electrodes placed on the skin. Stimulation was a 2 ms mono-polar square pulse with 5-sec. inter-stimulus interval, and the stimulus intensity was manually adjusted to individual pain detection threshold. The ERPs were recorded with a sample rate of 1000 Hz using an EEG amplifier (NuAmp, Neuroscan, El Paso, TX, USA) through a scalp electrode at Cz (international 10–20 system) with a frontal electrode as ground and an earlobe electrode as reference. The EEG amplifier received trigger signals from the stimulator, and data were collected on a computer. The recordings took place in a quiet, dim room with all unnecessary equipment turned off to minimize extraneous influences on the ERPs.

ERPs were averaged using a notch filter at 49–51 Hz with zero-phase-shift cut-off on 24 db/oct and were bandpass-filtered from 1 to 70 Hz with a zero-phase-shift filter with cut-off of 12 db/oct. Baseline was adjusted to the mean of −100 to 0 ms. Sixty-three ERPs were cleaned for artefacts by rejecting sweeps using EEG processing software (Neuroscan software v 4.3, Compumedics, El Paso, TX, USA). Two ERPs could not be cleaned adequately and were excluded on the justification that they had absolute values over ±200 μV [17].

Subjects were instructed in the use of a visual analogue scale and instructed to identify the pain detection threshold (the minimum intensity of a stimulus that is perceived as painful). Electrical stimulations of the median nerve were applied at increasing intensities until pain detection threshold was reached. ERPs were recorded at pain detection threshold from 60 stimuli repetitions. This procedure was repeated in a second run after approximately 10 min.

Data analysis. The pharmacodynamic parameters for placebo and buprenorphine effects were estimated using non-linear mixed effects modelling performed in the NONMEM software (Version 7.2.0) [18] with the Wings for NONMEM interface (version 720; http://wfn.sourceforge.net/). The R data analysis language (version 2.14.1) with ggplot2 library was used for graphical output and data manipulation [19]. The population approach was undertaken using the first-order conditional estimation procedure with interaction (FOCE-I). Pharmacodynamic models were developed in two steps. Firstly, a placebo submodel was developed on placebo data only, and the structure of the placebo submodels were then fixed in the development of the drug-response models.

For five of seven ERP sessions, a time-matching blood sample was collected, but for ERP sessions at 4 and 28 hr after dose (as well as for missing blood samples), buprenorphine drug concentrations had to be estimated for pharmacodynamic modelling. A cubic interpolating spline, developed previously to describe buprenorphine concentrations in the same data, was used to estimate concentrations, according to the equation [15]:

$$Y(t) = k_1 + k_2 \cdot t + k_3 \cdot t^2 + k_4 \cdot t^3$$

where Y is the concentration in the time interval between two observed concentrations and coefficients(k_{1-4}) were calculated for each subject and time interval using the 'spline' package of the R language. Splines were used rather than exponential functions as, the time-course of buprenophine plasma concentrations were complex and multi-peaked, confounding a description using standard compartment models.

Quantification of ERP. Three different metrics were used to quantify changes in the vertex potential of the ERPs.

Metric 1: PeakAmp. PeakAmp was defined and calculated as the difference between the identified N2 and P2 amplitude of the vertex potential. The subject mean N2 and P2 peaks were visually identified on the average of all ERPs recorded for each subject. N2 amplitudes were found by scanning a narrow interval of 50 ms around the position of subject mean N2. P2 was found for all subjects by scanning ERP for maximum value in the interval 150–300 ms. Scanning was programmed in the TCL language in the software Neuroscan. All identified peaks found by automatic scanning were validated visually and corrected manually if necessary.

Metric 2: PeakLat. PeakLat was defined as the latency of the identified N2 peak of the vertex potential. N2 latencies were found by scanning a narrow interval of 50 ms around the position of subject mean N2.

Metric 3: MeanAmp. MeanAmp was defined and calculated as the mean amplitude in the interval 116–152 ms. The MeanAmp metric, as opposed to PeakAmp, did not require subjective assessment of peaks, and was fully automated. The mean amplitude calculated in the interval 116–152 ms have reported in a previous study of ERPs in experimental pain electrically induced at the forearm [6], and thus might be potentially translatable between studies.

Placebo models. The placebo structural model was investigated among polynomials up to the 3rd order:

$$E_{\text{Placebo}} = E_0 + \theta_1 \cdot t + \theta_2 \cdot t^2 + \theta_3 \cdot t^3$$

where E_0 was baseline effect, t was time after dose and θ_{1-3} were estimable parameters [12]. 0th, 1st and 2nd order polynomials were tested by eliminating terms as necessary.

A combination of additive and proportional residual error was modelled. BSV was assessed to allow for interindividual variations on baseline and slope parameters. BOV was modelled on baseline to allow for different baselines between the treatment trials (1st or 2nd visit in the cross-over design). Normality of data and random effect distributions were assessed by Quantile–Quantile plots and distribution density plots. Box–Cox transformation was assessed on skewed random effects. Discrete covariates were assessed on baseline of bimodal data. Variations due to age and weight were assessed as continuous covariates.

Buprenorphine response model. The buprenorphine structural models were examined among linear, log-linear, $E_{\max}$ and sigmoidal $E_{\max}$ models as has previously been characterizing opioid effects [15,20]. Both direct and indirect pharmacodynamic models were tested, with indirect models having an effect compartment to describe any delays between plasma concentration and effect:

$$\frac{dC_e}{dt} = k_{e0} \cdot (C_p - C_e)$$

where C_p is the plasma concentration, C_e is the effect compartment concentration and k_{e0} is the effect delay rate constant. For direct models $C_e = C_p$.

Linear drug model:

$$E_{\text{drug}} = \text{SLP} \cdot C_e$$

where SLP is the drug effect slope and C_e is the effect compartment concentration.

Transformed log-linear drug model:

$$E_{\text{drug}} = \text{SLP} \cdot log(C_e + 1)$$

Addition of one to the concentration of the log-linear formula was used to achieve a model that was centred around baseline (E_0), and the concentrations were converted to units that ensured the model to remain log-linear in the relevant concentration interval.

Sigmoidal $E_{\max}$ model:

$$E_{\text{drug}} = \frac{E_{\max} \cdot C_e^{\gamma}}{EC_{50}^{\gamma} + C_e^{\gamma}}$$

where $E_{\max}$ is the maximum effect, EC_{50} is the concentration needed to achieve half the maximum effect. Models were examined both with and without the Hill coefficient γ [20].

Model selection and evaluation. Models were selected based on goodness of fit plots, residual and random effects distributions, parameter estimate precision and Akaike Information Criterion (AIC) [21,22]. A ΔAIC drop >2 was regarded as a critically better fit with empirical support for a complex model over a simpler model, when accounting for the number of parameters in the models. Two-hundred bootstraps were performed for evaluation of model stability using the Wings for NONMEM module (version 7) for NONMEM. Models were evaluated using Visual Predictive Checks based on 500 simulations performed in NONMEM and visualised using R.

Results

ERP recordings.

Of the 22 subjects who completed the study, three subjects were excluded from further data analysis due to insufficient signal to noise ratio, which prevented identification of the peaks. Figure 1 shows the average of all recorded ERPs (grand mean) for all included subjects with the visually identified peaks N2 and P2 of the vertex potential marked. The grey area-under-the-curve shows the relative position of the interval 116–152 ms that has previously been used to quantify opioid effects on ERPs after painful electrical stimulation of the forearm [6].

Peaks N2 and P2 were identified on average ERPs for each subject. Of the total number of ERPs for all time-points, 461 ERPs could be successfully identified by automatic scans in intervals of 50 ms around N2 to obtain PeakAmp and PeakLat data. For 13 ERPs, N2 was manually found within $\pm$25 ms of the automatically scanned interval. For eight ERPs, no N2 peak could be manually identified, and these were excluded from the PeakAmp and PeakLat data. MeanAmp was quantified for all ERPs. Figure 2 shows the relative position of peaks identified for PeakAmp and PeakLat analysis to MeanAmp in three representative examples.

Pharmacodynamic modelling.

Throughout the study period, buprenorphine concentrations were at least three times higher than the active metabolite norbuprenorphine, and given that the intrinsic analgesic effect of norbuprenorphine is one-fourth of buprenorphine in the rat

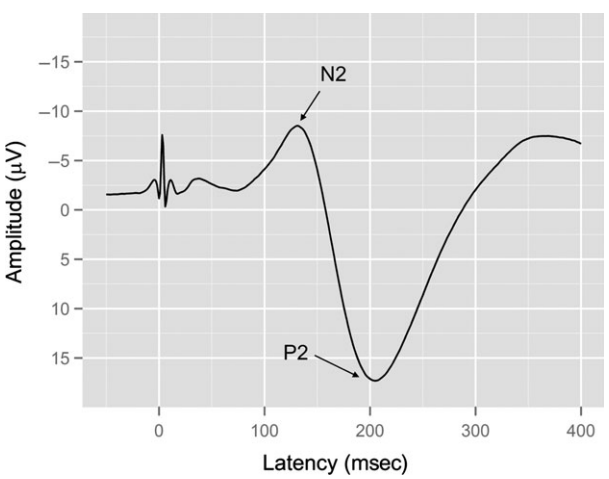

Fig. 1. Grand mean of all ERPs with denominations of the assumed vertex potential peaks N2 and P2.

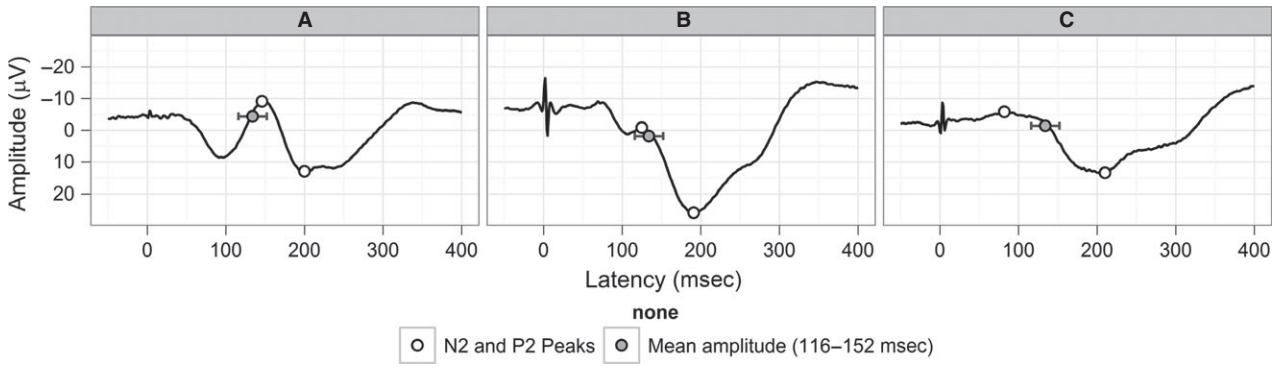

Fig. 2. Representative ERPs for three different subjects, recorded at baseline. (A) ERP with a clear negative–positive peak interpreted as the vertex potential. (B) ERP with multiple negative peaks present at latencies below 150 ms, which complicated identification of N2. (C) ERP with multiple overlapping negative peaks possibly responsible for producing a broad peak with shoulders around 120 ms. Automatically identified N2 and P2 peaks are plotted (white symbols). Mean amplitude in the interval 116–152 ms is plotted (grey symbols), with a grey band marking the interval 116–152 ms.

[23], it was decided to limit the concentration-effect analysis to buprenorphine.

PeakAmp model development.

The PeakAmp placebo data were best described by a 3rd order polynomial model. A critically better fit (lower ΔAIC) was obtained for the 3rd order polynomial compared with a linear model (ΔAIC = −7.0) and a 2nd order polynomial (ΔAIC = −2.6). BOV and Box-Cox transformed BSV were implemented on baseline, and BSV was implemented on the 1st order slope. All BSVs, BOVs and Box-Cox models were supported with improved ΔAIC and goodness of fit plots. A systematic deviation was investigated between values recorded for the two runs recorded at a 10-min. interval that constituted an ERP session. A discrete covariate (CovRun) was implemented to account for difference in baseline (E_0) between the first and second run. The implementation of CovRun on the PeakAmp model was supported by ΔAIC = −26.1 and improvements of goodness of fit plots. The PeakAmp drug response was best described by an indirect transformed log-linear model with BSV on the drug slope as seen in table 1. The indirect model provided a critically better fit than a direct model (ΔAIC = −29.1), and the log-linear model was better than a linear (ΔAIC = −21.5). E_{max} models were not selected despite critically better fits, as they proved unstable in bootstraps with parameter relative standard errors (%S.E.) of >1000%. The final PeakAmp model was evaluated using goodness of fit plots, visual predictive checks and bootstraps. Neither proportional nor additive error models could be omitted without a critical increase in ΔAIC. No improved fits were found when modelling age or weight as covariates on PeakAmp baseline or buprenorphine effects.

PeakLat model development.

The PeakLat placebo data were best described by a 2nd order polynomial model with BOV and BSV on the baseline. A critically better fit was obtained for this model compared with a linear model (ΔAIC = −4.8), but 3rd order polynomials did not improve fit further (ΔAIC = 1.3). A histogram of PeakLat data (fig. 3) revealed that subjects could be divided into two groups with distinct PeakLat distributions. A discrete covariate (CovGroup) was implemented to allow for a different PeakLat baselines (E_0) between early and late groups. The appropriateness of this covariate was supported by improvements in ΔAIC and goodness of fit plots.

The PeakLat drug response to buprenorphine was best described by a direct transformed log-linear model with BSV on the drug slope as seen in table 1. The fit improved critically with a log-linear *versus* a linear model (ΔAIC = −8.6). No indirect log-linear model converged successfully. As for PeakAmp, Emax models were not selected due to parameter instability.

The final PeakLat model was evaluated using goodness of fit plots, visual predictive checks and bootstraps. Neither proportional nor additive error models could be omitted without a critical increase in ΔAIC. No age- or weight-related effects were found on PeakLat.

MeanAmp model development.

The MeanAmp placebo data were best described by a 2nd order polynomial model with BOV and Box-Cox transformed BSV on baseline and BSV on the 1st order slope. Second order models provided better fit than linear models (ΔAIC = −14.7), but there was not a critically improved fit with a 3rd order model (ΔAIC = −1.4). Similar to the PeakAmp model, a covariate (CovRun) was implemented on baseline (E_0) to account for difference between the first and second runs in an ERP session with improved fit (ΔAIC = −22.5) and goodness of fit plots.

The MeanAmp drug response was also best described by an indirect transformed log-linear model with BSV on the drug slope as seen in table 1. Log-linear models provided critically better fits than linear models (ΔAIC = −15.3), and no indirect log-linear model converged successfully. As for PeakAmp and PeakLat, E_{max} models were not selected due to parameter instability.

Table 1.
Population parameter estimates and % standard error (%S.E.) produced by NONMEM of the three pharmacodynamic models. All population variabilities (expressed as standard deviation ω) were additive unless specified.

	PeakAmp			PeakLat			MeanAmp		
	Units	Estimate	%S.E.	Units	Estimate	%S.E.	Units	Estimate	%S.E.
Placebo Model parameters									
Baseline	μV	24.9	18.2	ms	138	2.1	μV	−5.43	25.6
θ_1	μV/hr	−0.217	48.4	ms/hr	−0.0622	49.7	μV/hr	0.0445	28.3
θ_2	$\mu V/hr^2$	0.00488	36.7	ms/hr^2	0.000349	51.6	$\mu V/hr^2$	−0.000338	22.0
θ_3	$\mu V/hr^3$	−0.0000221	36.7	–	–	–	–	–	–
$\omega_{BSV\ (baseline)}$	μV	10.1	108.8	–	0.0763[1]	32.4	μV	5.11	45.6
$\omega_{BOV\ (baseline)}$	μV	6.10	80.9	–	0.0321[1]	37.4	μV	1.85	48.3
$\omega_{BSV\ (slope1)}$	μV/hr	0.158	181.7	–	–	–	μV/hr	0.0136	44.5
CovRun	–	0.855	2.7	–	–	–	–	0.750	7.8
CovGroup	–	–	–	–	0.634	4.0	–	–	–
BoxCox	–	0.0759	44.5	–	–	–	–	−0.108	29.6
Drug model parameters									
Drug slope	–[2]	0.501	115.0	–[2]	0.266	133.5	–[2]	−0.211	61.6
K_{e0}	hr	0.442	85.5	–	–	–	–	–	–
$\omega_{BSV\ (Drug\ slope)}$	–	1.45	49.3	–	1.136	35.8	–	0.473	51.8
RUV									
$\varepsilon_{proportional}$	%	8.82	98.1	%	3.30	66.9	%	9.20	55.2
$\varepsilon_{additive}$	μV	3.54	53.7	ms	3.46	106.7	μV	1.80	10.6

BSV, between-subject variability; BOV, between-occasion variability, RUV, residual unexplained variability; CovRun, Discrete covariate between first and second ERP repetition, modelled on baseline; CovGroup, Discrete covariate between early and late peaks, modelled on baseline; BoxCox, Box-Cox value, modelled on baseline BSV.
[1]Exponential BSV and BOV.
[2]Slope to the log of transformed buprenorphine concentration, which can be regarded as unitless.

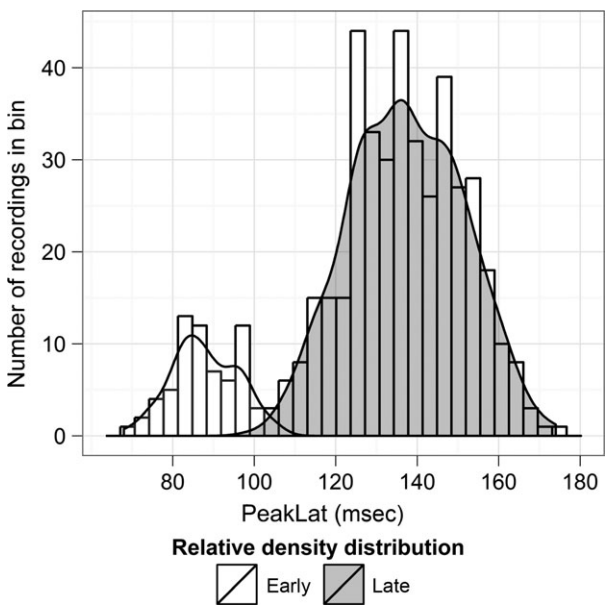

Fig. 3. Histogram of PeakLat data. Relative density distributions is grouped in subjects identified as having early N2 (white) and late N2 (gray).

The final MeanAmp model was evaluated using goodness of fit plots, visual predictive checks and bootstraps. Neither proportional nor additive error models could be omitted without a critical increase in ΔAIC. No age- or weight-related effects were found on MeanAmp.

Pharmacodynamic model predictions of effects on ERP.
PeakAmp, PeakLat and MeanAmp final models all conformed to the model selection and validation criteria. Parameter estimates and %S.E. for the final three pharmacodynamic models are presented in table 1.

All models contained both BSV and BOV on baseline (E_0). The BSV as a proportion of the total variance of BSV + BOV was calculated as 73.3% for PeakAmp, 85.0% for PeakLat and 88.4% for MeanAmp.

Figure 4 shows the visual predictive checks for each model, and fig. 5 shows the individual predicted *versus* observed response.

All models included a logarithmic buprenorphine concentration-effect relationship with a BSV on the slope parameter. Only PeakAmp converged successfully with a modelled effect delay. For all models, the population mean effect slope had a 90% confidence interval including 0, as calculated from the estimated %S.E. from NONMEM. This suggests that no population drug effect (i.e. slope = 0) was a plausible description of the data. The individual and population effects in the relevant concentration interval are visualized in fig. 6.

Discussion

A robust procedure is described for development of non-linear mixed effects models of placebo and drug effects on ERPs in experimental pain using ΔAIC and goodness of fit plots and visual predictive checks as model development criteria. Three models were developed on different ERP metrics to adequately

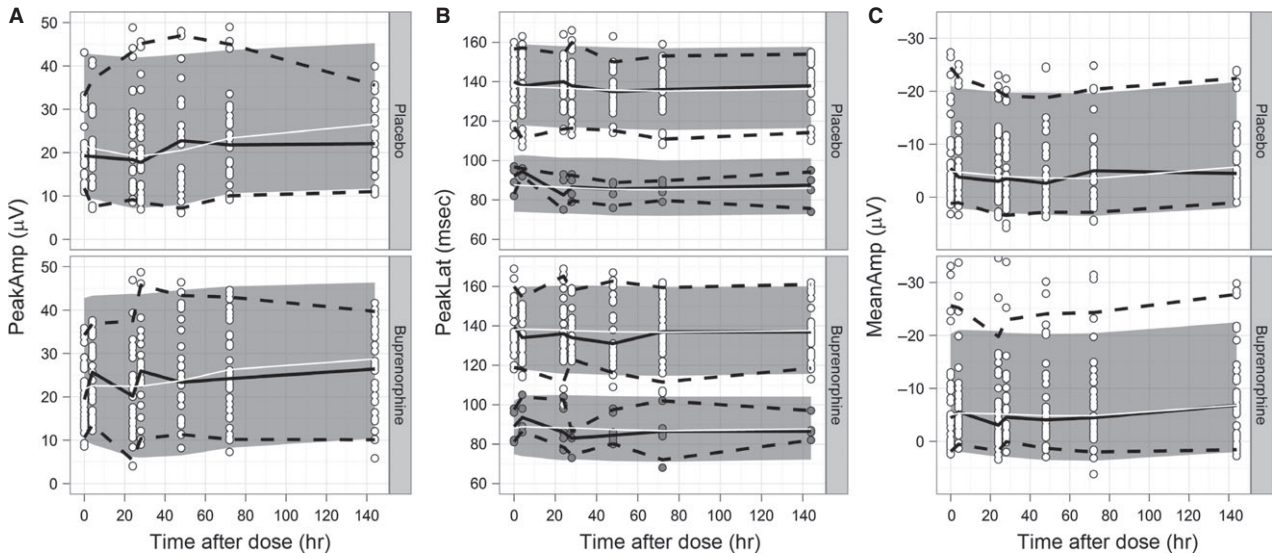

Fig. 4. Visual Predictive Checks for pharmacodynamic models (A) PeakAmp, (B) PeakLat and (C) MeanAmp. Visual Predictive Checks are shown separately for placebo (top) and buprenorphine (bottom) data. Observed data is plotted with median (black lines) and 5th and 95th percentile (dashed lines). The model predicted median (white lines) are shown along with the 90% prediction intervals (grey shade). Visual Predictive Checks for PeakLat have been divided into two groups: early (white symbols) and late (gray symbols). This reflects the covariate implemented on baseline to separate data with distinct distributions.

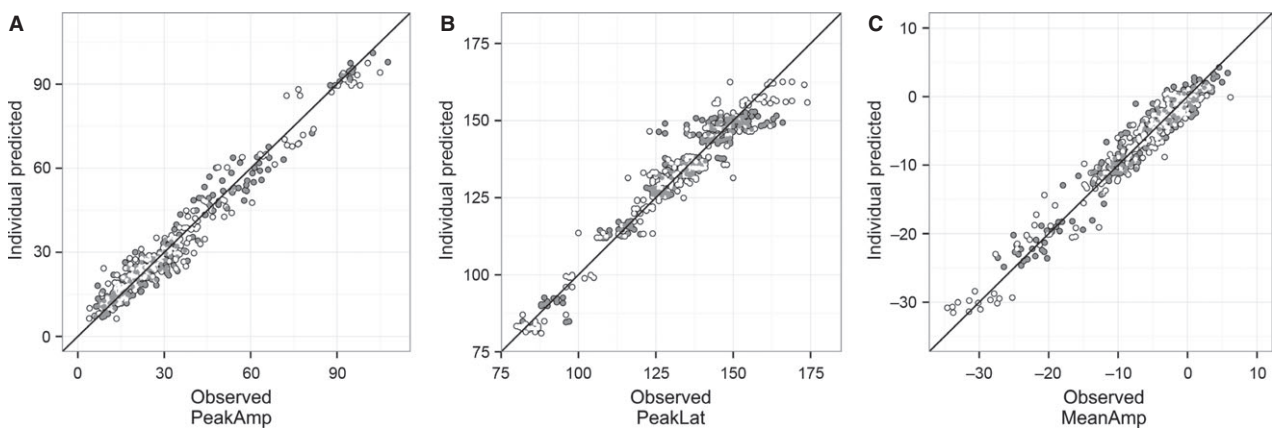

Fig. 5. Individual predicted *versus* observed response for (A) PeakAmp, (B) PeakLat and (C) MeanAmp. Response data are shown for placebo (grey) and buprenorphine (white).

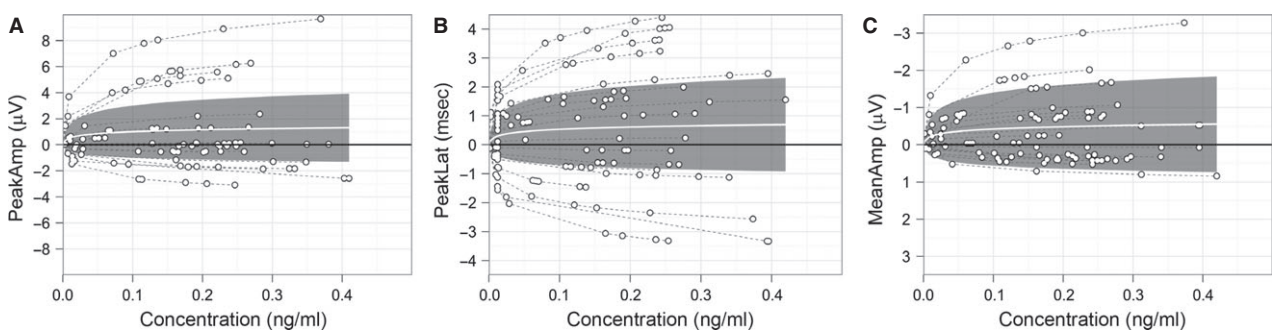

Fig. 6. Predicted buprenorphine effect for (A) PeakAmp, (B) PeakLat and (C) MeanAmp, when corrected for baseline and random errors. Individual predicted effects are marked with dashed lines. Population mean is shown (white line) with 90% confidence interval (grey band). The 90% confidence interval around drug effect slope in all instances included zero.

describe the time course of ERP data from both placebo and buprenorphine arms of the study.

Impact of modelling placebo and baseline variability. All structural developed placebo models were either polynomial, quadratic (PeakLat and MeanAmp models) or cubic (PeakAmp model) with individual variability (modelled as BSV) in slopes. The placebo models indicate that ERPs develop systematically over time, which may both be a natural development over time and a direct result of placebo effects.

The developed placebo-model descriptions of placebo time-course was considered a significant improvement with substantial empirical support compared with more simple models (constant or linear), as based on the reduction in AIC ($\Delta AIC < -10$) [21]. This is considered empirical evidence that conducting placebo-controlled cross-over studies is valuable in experimental pain ERP studies. Failure to take into account systematic changes in response over time have been reported to lead to false conclusions about drug effects [12].

It was seen that baseline in measures related to amplitude (both PeakAmp and MeanAmp) were not normally distributed over individuals, but needed to be transformed using a box–cox transformation for adequate description in the models. It was evident that PeakLat data were not normally distributed, but bi-modal in nature and that subjects could be separated into two groups, one with early latency N2 and one with late latency N2. Deviations to normality could have hampered data analysis, but pharmacodynamic modelling allowed for covariates implementation and transformations to account for this. Furthermore, the BOV model in all models described that significant (based on ΔAIC) variability in ERP was present between first and second treatment in the cross-over study. Together, these findings add to understanding of the complexity of ERP data, but also suggest that simplified data analysis not accounting for data and variance distributions is suboptimal [6,9].

With non-linear mixed effects modelling as presented in this paper, it was possible to develop placebo models that accounted for the placebo and non-normal distributions as discussed. Assessed by visual predictive checks, the developed placebo models provided adequate descriptive abilities of baseline and placebo-time course to be suitable for placebo correction in the analysis of buprenorphine effects.

Identification of variability sources to improve pharmacodynamic models. Large variability was observed in ERP data, as was expected from descriptions of previous literature [4]. However, the developed pharmacodynamic models revealed that the major part of the variability could be explained as variation within subjects or study occasion on baseline (modelled as BSV and BOV). The combined (explained) variance of population parameters on baseline (BSV + BOV) was in all metrics more than 8 times higher than the combined variance of unexplained residual error (additive and proportional) when calculated at baseline mean levels. From the visual predictive checks and random effects models, it was apparent that variance was relatively constant over time. BSVs were in all cases as large compared with BOVs as the BSV proportions were calculated to be between 73–88% of the total population parameter variance (BSV + BOV) on baseline for the three models.

Together, these findings suggest that ERPs are relatively reproducible especially within individuals, despite the large variability in data. Improvements in the parameter estimation and hence outcome of ERP studies can be expected if systematic causes of variation between individuals or different study settings can be identified and implemented as covariates. One such covariate was found in this study, as a discrete covariate (CovRun) was successfully implemented that described a significant reduction in baseline of vertex potential amplitude (PeakAmp and MeanAmp) between the first and the second of two replicate run with approximately 10-min. interval. This reflected a well-known phenomenon in ERPs described as habituation [7], where the second of two ERPs conducted in a short interval has reduced amplitude. Implementing this led to a reduction in random effects and hence improved model strength.

The intensity of applied electrical stimulus is known to influence the ERP under various conditions [3,4], and would be interesting to study as a covariate in the placebo models. However, the applied stimulus intensity was not available in this study, and so the magnitude of any potential relationship between this and ERP could not be investigated. In future studies of ERP with variable stimulus intensity, researchers are encouraged to study the continuous effect of stimulus intensity on ERPs. Potentially circadian rhythm may also affect the baseline ERP response, and while this could not be investigated in this study with only daily ERPs, it would be an interesting topic for future studies. Also covariates due to demographic variation might potentially improve models of ERP, but in this study, subjects were homogenous regarding age, weight and height as neither parameter explained any significant variations in baseline, placebo or drug effects, when modelled as covariates.

Quantification of ERP and buprenorphine effect models. PeakAmp and PeakLat quantified the amplitude and latency of ERP vertex potentials in a procedure that resembled many previous studies on opioid effects on ERP [7–9]. However, in this study, it was found that a bi-modal distribution of subjects was present based on the latency of the identified N2. It was noted that for most subjects, there was a tendency towards two distinct peaks around the expected latency of N2. It was not in all cases possible to identify a minimum value for both peaks, as they overlapped. This is consistent with previous findings that electrical stimuli can result in multiple negative peaks that are not necessarily related to the vertex potential [3]. The bi-modal distribution might therefore signify that for some subjects, a peak not related to the vertex potential might have been quantified for PeakAmp and PeakLat. As an alternative to measure amplitude, the mean amplitude (MeanAmp) was quantified in a specific interval as proposed by a previous study where ERP was recorded after painful electricity was applied to the forearm [6]. This quantification was simple to

perform, did not require a minimum value in the selected interval, did not require manual evaluation of the identification and did not produce bi-modal distributions. It was found that PeakAmp provided a less noisy metric than MeanAmp, as both the additive error component relative to baseline as well as the proportional error was lower for PeakAmp. This is in line with the high signal-to-noise ratio of N2 to P2 peak to peak amplitude, which is often presented as the rationale for using this metric [3,4]. However, it was not possible to evaluate which metric had the best relationship with magnitude of the actual ERP vertex potential. More advanced quantification methods could have been chosen [16], but the three simple methods applied sufficed to demonstrate the impact of accounting for placebo effects and baseline variation and support the use of pharmacodynamic modelling.

The models developed were in general consistent for all three metrics used to analyse ERPs for effects in vertex potential amplitude and latency. Direct and indirect log-linear effect models were selected based on empirical fit from a range models, as no related models of buprenorphine effects on ERP were found in the literature. Practical and ethical considerations limited the pharmacodynamic sampling to seven ERP sessions over 0–144 hr. These data did not allow stable fits of advanced effect models such as sigmoidal Emax and biophase models. This does not preclude that the actual relationship between ERPs and opioids may be more advanced than the direct or indirect log-linear relationship found in this study. It is possible that more frequent sampling or sampling post-dosing (beyond 144 hr) may have revealed more clear time-dependent tendencies.

The lack of clear drug effects of buprenorphine on ERPs is in contrast to both buprenorphine effects on other experimental pain metrics [15] and opioid effects on the vertex potential amplitude of ERPs in experimental pain [6–9]. This lack of effect was not due to a subtherapeutic dosing of buprenorphine, as the same dose in the same population showed significant effect in models of bone-associated pain, heat pain, nerve growth factor induced soreness and cold pressor pain models [15]. However, it questions the usefulness of pain detection threshold adjusted ERPs as a measure of opioids effects. Previous studies of opioid effects on ERPs [6–9] all used constant stimulus intensities. In these studies, increased opioid concentration has resulted in reduced pain intensity. However, in this study, stimulus intensity was adjusted to achieve the same pain (pain detection threshold), and hence increased buprenorphine concentration did not lead to reduced pain, but will likely have affected the stimulus intensity. Stimulus intensity was not available for this study. However, the pharmacodynamic models developed and the methods described in this study provide a framework for describing also variable stimulus intensity if this is available from future trials.

Conclusion

This paper demonstrates the importance of correcting for placebo effects and non-normal distributions in ERP data of experimental pain. A robust method is presented for developing pharmacodynamic models that adequately described ERP placebo and buprenorphine data for 19 healthy volunteers after experimental stimulus of the median nerve at pain detection threshold. It is concluded that population pharmacodynamic modelling is a promising tool to identify sources of variability when studying ERP in experimental pain. Future studies should focus on identifying and describing variations in ERP between individuals and study conditions.

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